JOURNAL OF CLINICAL MICROBIOLOGY, Feb. 2010, p. 427–432 Vol. 48, No. 2
0095-1137/10/$12.00 doi:10.1128/JCM.01589-09

Morphologic and Phylogenetic Characterization of *Conidiobolus lamprauges* Recovered from Infected Sheep[▽]

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Received 17 August 2009/Returned for modification 5 October 2009/Accepted 1 December 2009

***Conidiobolus lamprauges*, a soil and plant entomophthoralean fungus, has been reported only in a horse and, more recently, in sheep with rhinopharyngeal entomophthoramycosis. Thus, little information is available to enable proper identification of this pathogen and its differentiation from other saprotrophic and pathogenic *Conidiobolus* species. Using classical mycological tools and molecular methodologies, we report for the first time the taxonomic and phylogenetic description of three *C. lamprauges* isolates recovered from sheep with rhinopharyngeal entomophthoramycosis. The distinctive clinical and pathological features of *C. lamprauges* are compared with those of other *Conidiobolus* spp. affecting sheep, as well as with those of the stramenopilan ovine agent *Pythium insidiosum*. The comparative morphological attributes of *Conidiobolus* spp. are also diagramed; along with the sequence data generated, they should assist laboratories in the identification of these uncommon species.**

Several species of entomophthoralean fungi in the genera *Basidiobolus* and *Conidiobolus* have been reported as the etiologic agents of subcutaneous entomophthoramycosis in apparently healthy hosts (13, 21). In contrast, opportunistic members of the Mucorales have rarely been found causing subcutaneous infections in healthy mammalian hosts (20). A difference between pathogenic species of the Mucorales and Entomophthorales is that the latter group of pathogens triggers eosinophilic granulomas with the Splendore-Hoeppli phenomenon around coenocytic hyaline hyphae (13, 21), a unique characteristic shared only with *Pythium insidiosum*, a fungus-like zoosporic stramenopile pathogen (16, 29). The majority of subcutaneous infections caused by entomophthoralean fungi involve *Basidiobolus* spp., *Conidiobolus coronatus*, or *Conidiobolus incongruus* (13, 21). These fungal pathogens have been studied extensively, and their morphological characteristics are readily available (13). This is not the case for *Conidiobolus lamprauges*, which has been reported only once previously, from a horse (10). Thus, limited data are available for the proper identification of this newly described *Conidiobolus* sp. recovered in culture from clinical samples.

Outbreaks of sheep rhinitis involving entomophthoralean fungi are frequently encountered in Brazil (1, 22, 26). The disease occurs in two different clinical forms: (i) a rhinofacial infection affecting the nose vestibule, mucoepithelial junction, and upper lip and (ii) a rhinopharyngeal infection affecting the ethmoidal region, turbinate bones, paranasal sinuses, hard and soft palates, and pharynges (1, 22). Interestingly, *Conidiobolus coronatus* and other *Conidiobolus* spp. have been isolated in cases of nasopharyngeal entomophthoramycosis, whereas *Pythium insidiosum* is usually recovered in cases of rhinofacial pythiosis (22, 24, 26). This study describes in detail, for the first time, the taxonomic and phylogenetic features of three *C. lamprauges* strains isolated in cases of rhinopharyngeal entomophthoramycosis in three Brazilian sheep.

MATERIALS AND METHODS

Strains. Tissue samples collected from three sheep with rhinopharyngeal clinical signs of rhinitis were cut into pieces (diameter, 4 to 5 mm) and placed on 2% Sabouraud dextrose agar (SDA) plates. The inoculated plates were then incubated at 37°C or 25°C for a week. The isolates recovered from the infected sheep were macroscopically and microscopically evaluated by placing pieces of their colonies (thickness, 2 mm; diameter, 2 mm) on lactophenol blue (2 ml phenol, 2 ml lactic acid, 4 ml glycerol, 2 ml H_2O). The original strains on SDA were also subcultured on 2% potato dextrose agar (PDA) (20 g potatoes, 1.0 g dextrose, 2.0 g agar, and 100 ml H_2O), and their macroscopic and microscopic morphological features were evaluated. For the DNA extraction protocol, the three *C. lamprauges* strains studied—BLDCL-01 (cited in reference 22), BLDCL-02, and BLDCL-03—were maintained at room temperature on SDA, and then pieces of their colonies (thickness, 4 mm; diameter, 4 mm) were subjected to one of two procedures. In the first, the pieces were transferred to 250-ml flasks containing 100 ml of 2% Sabouraud dextrose broth (SDB) and were then incubated at 37°C with rotation at 150 rpm for 5 days. In the second, the pieces were placed on SDA plates, and their hyphae were removed from the surface of the agar after 5 days of incubation at 37°C.

DNA extraction, PCR protocol, and sequence analysis. After incubation, the cell mass obtained from SDB and/or SDA cultures was killed with Merthiolate (0.02%, wt/vol). The resulting cell mass was placed in a mortar and ground under liquid nitrogen. The DNA from the hyphae was treated with sodium dodecyl sulfate and proteinase K digestion and was then extracted with phenol, chloroform, and isoamyl alcohol (Sigma, St. Louis, MO). The 18S small-subunit (SSU) ribosomal DNA (rDNA) gene was amplified by hot-start PCR using the universal

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▽ Published ahead of print on 9 December 2009.

TABLE 1. Strains and GenBank accession numbers of the 18S SSU rDNA sequences used in this study

Strain	Origin	GenBank accession no.
Basidiobolus haptosporus CBS 358.65 (ATCC 16109)	Human, Uganda	AF113413
Basidiobolus ranarum NRRL34594	Unknown	AY635841
Basidiobolus microsporus ARSEF265	Lizard dung	AF368505
Conidiobolus brefeldianus ARSEF452	Unknown	AF368506
Conidiobolus firmipilleus ARSEF2731	Insects	AF368507
Conidiobolus coronatus		
F943	Unknown	D29947
NRRL28638 (CBS 209.66, ATCC 28846)	Plant detritus	AF113418
Unknown	Unknown	AF296753
Conidiobolus incongruus NRRL28636 (CBS 108.84)	Human, Thailand	AF113419
Conidiobolus lamprauges		
NRRL28637 (CBS 153.56, ATCC 12592)	Plant detritus	AF113420
ARSEF2338	Environmental	AF296754
BLDCL-01	Sheep, Brazil	GQ478279
BLDCL-02	Sheep, Brazil	GQ478280
BLDCL-03	Sheep, Brazil	GQ478281
Conidiobolus osmodes ARSEF79	Insects	AF368510
Conidiobolus pumilus ARSEF453	Plant detritus	AF368511
Conidiobolus rhysosporus ARSEF448	Plant detritus	AF368512
Conidiobolus thromboides ARSEF115	Insects	AF052401

NS1 5′-GTAGTAATATGCTTGTCTC-3′ and NS8 5′-TCCGCAGGTTCACA ACGGA-3′ primers (8). The PCR temperature cycling parameters were as follows: 10 min at 95°C for the hot start and 1 min at 95°C for subsequent cycles, annealing for 1.0 min at 60°C, and elongation at 72°C for 2 min. This procedure was repeated for 40 cycles, followed by a final elongation of 2 min at 72°C. The amplicons were ligated into the pCR 2.1-TOPO vector (Invitrogen, Carlsbad, CA), purified, and then sequenced using BigDye Terminator chemistry in an ABI Prism genetic analyzer, model 310 (Perkin-Elmer, Foster City, CA).

Phylogenetic analysis. The DNA sequences of the strains evaluated were aligned with 15 other fungal sequences available in the database using CLUSTAL X (32) analysis (Table 1), and their alignments were inspected visually. The gaps generated were treated as missing data. The 18 fungal DNA sequences were analyzed phylogenetically by the neighbor-joining (NJ) method, parsimony, and minimum evolution with multiple-hit correction by Kimura's 2-parameter model using MEGA4 software (30). Support for internal branches was assessed by using 1,000 bootstrapped data sets.

Nucleotide sequence accession numbers. The 18S SSU rDNA sequences obtained in this study for *C. lamprauges* BLDCL-01, BLDCL-02, and BLDCL-03 have been deposited in GenBank under accession numbers GQ478279 to GQ478281.

RESULTS

***Conidiobolus lamprauges* strains.** The three isolates were identified as *Conidiobolus* species based on their microscopic features (see below). The three strains, BLDCL-01, BLDCL-02, and BLDCL-03, developed fast-growing isotropic, pale colonies in the primary culture on SDA at 25°C and 37°C (Fig. 1). On this medium, within the first 3 days of incubation at either temperature, flat membranous colonies with a smooth, glabrous, and waxy appearance developed (Fig. 1A, inset). Older colonies (3 days old and older) became powdery in appearance, with short aerial mycelia, and developed radiate folds from the centers of the colonies (Fig. 1A and B). On PDA at 25°C and 37°C, colonies were thin, flat, and smooth, with folds at the center of the colonies (data not shown). The colonies of the three strains studied developed faster on SDA and PDA at 37°C (diameters, 20 mm in 24 h and 55 mm in 72 h) than at 25°C (diameters, 15 mm in 24 h and 45 mm in 72 h).

Microscopically, the three colonies evaluated on SDA showed hyaline, coenocytic, ribbon-type, unbranched hyphae measuring 5.0 to 10.0 (±2 to 4) μm in diameter, with occasional septa, usually on the distal parts of the hyphae (Fig. 2A). Immature zygospores developed after the encounter of two hyphal segments, producing swelling in the contact area and later becoming globose, with several internal vacuoles (data not shown). Numerous smooth globose to subglobose ripe zygospores, 10.0 to 18.0 μm in diameter, were observed on primary SDA cultures for strain BLDCL-01 (Fig. 2A and B), but not for the other two strains. The internal cell walls of the mature zygospores were 1.0 to 4.0 μm in diameter (±0.5 to 1.0 μm; $n = 50$). Some of the mature zygospores contained a readily detectable large globule at the center, with some space between the internal globule and the cell wall (Fig. 2B). The ability of strain BLDCL-01 to develop zygospores on SDA (primary cultures) was lost after the first subculture on the same medium. On PDA, all strains evaluated developed nu-

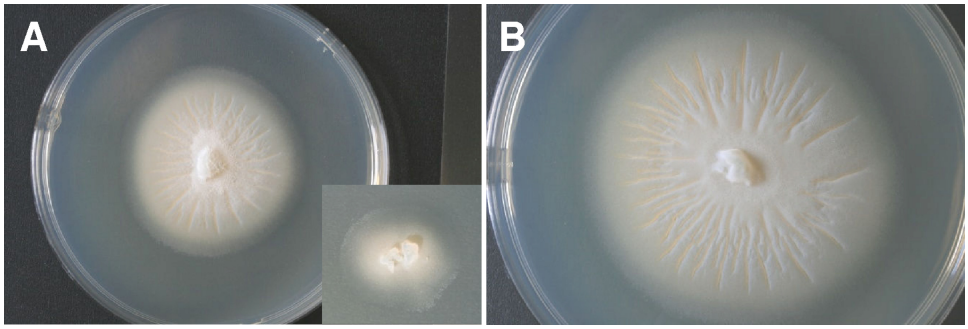


FIG. 1. *Conidiobolus lamprauges* strain BLDCL-01 on SDA. (A) A smooth, glabrous, waxy 3-day-old colony of *C. lamprauges* at 37°C. (Inset) The same colony after 24 h of incubation at 37°C. (B) The same colony after more than 6 days of incubation at 37°C. Older colonies are characterized by the development of powdery structures with short aerial mycelia discharging papillate conidia, with folds radiating from the centers of the colonies.

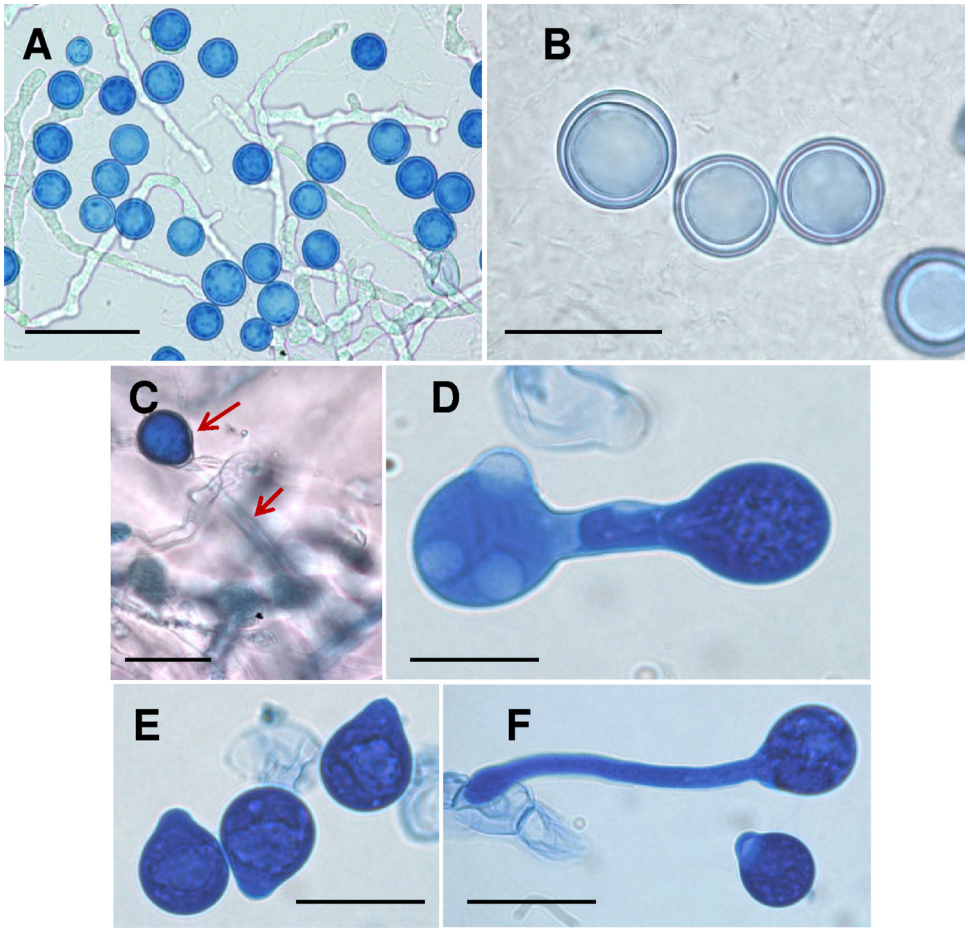


FIG. 2. (A) Hyaline, coenocytic hyphae of *Conidiobolus lamprauges* strain BLDCL-01, with numerous smooth, globose mature zygospores, in lactophenol blue. Bar, 40 μm. (B) Close-up of smooth, mature *C. lamprauges* zygospores on PDA medium, with some space between the internal globules and the cell wall. Bar, 20 μm. (C to F) Different features of the asexual papillate globose conidia observed on PDA. (C) A single hyphal segment (lower red arrow) with a conidium (upper red arrow) before it was forcibly discharged. Bar, 25 μm. (D) Migration of the cytoplasm from the primary papillate conidia into secondary sporophores (replicative conidia). This type of conidium is commonly found in other *Conidiobolus* species, but the sizes of the structures might differ. Bar, 15 μm. (E) Primary asexual globose papillate conidia of *C. lamprauges*. Bar, 20 μm. (F) One of the *C. lamprauges* asexual conidia developing a germ tube that eventually becomes a hypha, used to colonize new environments. Bar, 20 μm.

merous zygospores identical to that described for isolate BLDCL-01 on SDA (Fig. 2A and B). Asexual papillate globose conidia were observed on PDA in the three isolates studied (Fig. 2C to F). The asexual conidia measured 15.0 to 20 (±3.0) μm in diameter and developed in a single hyphal segment from which they were forcibly thrown off (Fig. 2C, red arrows). The cells wall of the conidia were smooth and lacked the layered features in the inner cell wall characteristic of mature zygospores (Fig. 2C to E). The ejected asexual conidia each possessed a rounded apiculate basal papilla, and some of them developed germ tubes a day or two after discharge (Fig. 2E and F). On PDA medium, migration of cytoplasm from the primary papillate conidia into secondary sporophores was also observed in the three *C. lamprauges* strains studied (Fig. 2D).

Phylogenetic analysis of *C. lamprauges* from sheep. The GenBank accession numbers of the 18S SSU rDNA sequences determined in this study are given in Table 1. Few nucleotide mismatches were found within the three *C. lamprauges* DNA sequences from sheep. High-stringency BLAST (http://www.ncbi.nlm.nih.gov/) analysis of these three 18S SSU rDNA sequences (1,740 bp) showed strong identity with the DNA sequences of two environmental strains of *C. lamprauges*, one from a Swiss study (GenBank accession number AF296754) (99% identity) and one, strain CBS 153.56 (AF113420), from Virginia (92% identity), followed by *Conidiobolus osmodes* (90% identity), *Conidiobolus thromboides* (90% identity), and other closely related members of the Entomophthorales. By the use of *Basidiobolus* species as an outgroup, the phylogenetic trees constructed by the three phylogenetic methods in this study (neighbor joining, minimum evolution, and parsimony) showed no significant topological conflicts (Fig. 3). The three *C. lamprauges* DNA sequences recovered from sheep and the two environmental *C. lamprauges* DNA sequences available in the database clustered together, forming a strongly supported monophyletic taxon (100% NJ bootstrap and 100% parsimony bootstrap). The *C. lamprauges* taxon is connected by a long branch to *C. osmodes*, *C. thromboides*, *Conidiobolus pumilus*, and *Conidiobolus rhysosporus*, which, in turn, are sister taxa to the remaining *Conidiobolus* DNA sequences in this study (Fig. 3). These remaining sequences cluster into two

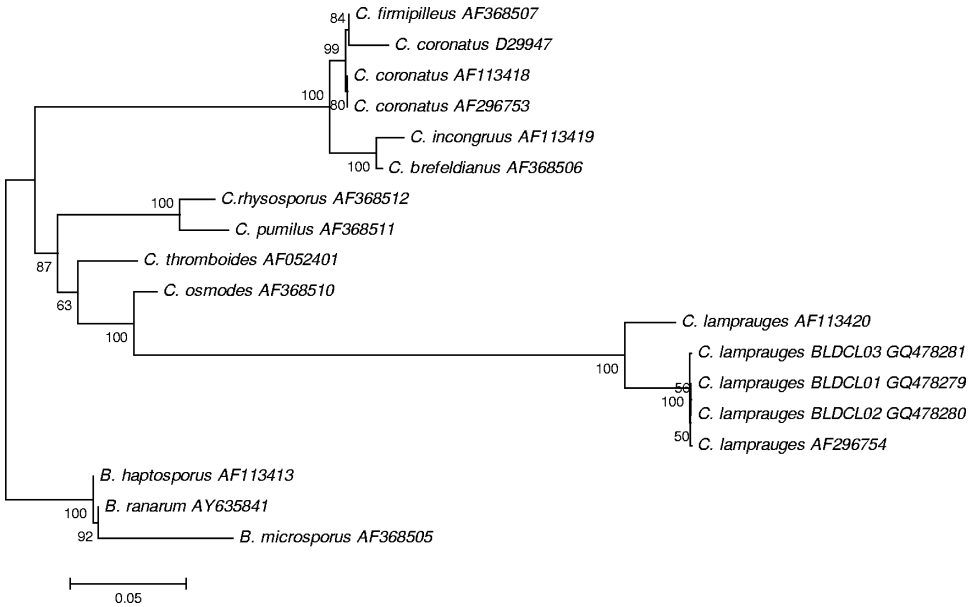


FIG. 3. Neighbor-joining tree of aligned 18S SSU rDNA sequences of the 3 *Conidiobolus lamprauges* strains from sheep and 15 other Entomophthoramycetes sequences (Table 1). The percentages of 1,000 bootstrap-resampled data obtained by neighbor-joining analysis are given on the branches. The DNA sequences of *C. lamprauges* strains BLDCL-01 (from reference 22) (GenBank accession number GQ478279), BLDCL-02 (GQ478280), and BLDCL-03 (GQ478281), recovered from three Brazilian sheep in this study, clustered with those of two environmental *C. lamprauges* strains available in the database. In this tree, the five *C. lamprauges* DNA sequences clustered in a monophyletic group connected by a long branch to *C. osmodes*, *C. thromboides*, *C. pumilus*, and *C. rhysosporus*. This taxon, in turn, formed a sister group to the other *Conidiobolus* species used in this study. The DNA sequences of three *Basidiobolus* species were used as the outgroup. The bar represents 0.05 substitution per nucleotide.

small, well-supported sister groups (100% NJ bootstrap and 100% parsimony bootstrap), with three *C. coronatus* strains plus *Conidiobolus firmipilleus* in one clade and *C. incongruus* plus *Conidiobolus brefeldianus* DNA sequences in the other (Fig. 3).

DISCUSSION

Humans (2, 9, 20, 31, 35) and several species of lower animals, including deer (14, 28), dolphins (17), horses (15, 27, 37), llamas (7, 18), primates (23), and sheep (3, 12, 19, 26), have been reported with subcutaneous entomophthoramycoses of the nostrils, facial areas, and orbit caused by *Conidiobolus* species. Reports of *C. coronatus* (26), *C. incongruus* (3, 12, 19), and the stramenopilan fungus-like pathogen *P. insidiosum* (22, 24, 29) causing similar clinical signs in sheep suggest that in this mammalian species, multiple etiologies can be expected. The fact that *C. lamprauges* has been isolated in rhinopharyngeal cases and *P. insidiosum* in rhinofacial cases (22) should be further investigated. We believe that this clinical feature could be used as an aid in the differential diagnosis of entomophthoramycoses caused by these species.

Since the first description of *C. lamprauges* from environmental samples (5), only two reports of the species, affecting a horse and sheep, respectively, have ever been published (10, 22). Indeed, a review of the literature showed that most cases of *Conidiobolus* infection in humans and lower animals involved either *C. coronatus* (7, 13, 27, 37) or *C. incongruus* (2, 3, 9, 14, 19, 25). However, due to the limited information on the proper identification of *C. lamprauges* in the laboratory, it is quite possible that by use of classical taxonomic tools only, some *Conidiobolus* species might have been misidentified. For instance, Morris et al. (19) recently described *C. incongruus* in sheep. A careful review of the microscopic structures in their culture, including the formation of zygospores, showed that the morphological features of the fungus described in their report are similar to those encountered with *C. lamprauges*. Likewise, Hernandez et al. (9) and Sharma et al. (25) identified their fungal isolates from human patients as *C. incongruus*, based only on the production of papillate conidia and the absence of villose conidia. Thus, the lack of a laboratory guide for the taxonomic identification of three pathogenic *Conidiobolus* species has been problematic. To resolve this drawback, we have constructed a table listing several morphological differences and similarities between the three known pathogenic *Conidiobolus* species (Fig. 4), which can be used by laboratory workers dealing with clinical isolates of entomophthoramycetous fungi. According to Fig. 4, *C. coronatus* in culture can be distinguished from *C. lamprauges* and *C. incongruus* by the production of villose conidia and the absence of zygospores on PDA. Likewise, *C. lamprauges* and *C. incongruus* can be properly distinguished from each other by the morphological features of their mature zygospores (Fig. 4). Although human cases of *C. lamprauges* have yet to be reported, Fig. 4 could also aid in the proper identification of *C. lamprauges* from putative cases of entomophthoramycosis in humans.

Molecular methodologies have proved to be a valuable diagnostic tool in identifying clinical isolates and a variety of etiologic agents directly from infected tissues (14, 36). Using this approach, Madson et al. (14) reported the presence of *C.*

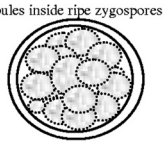
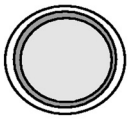
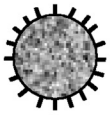
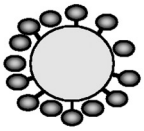
Structures in culture	*Conidiobolus coronatus*	*Conidiobolus incongruus*	*Conidiobolus lamprauges*
Zygospores	Absent	Present in PDA medium. 15 to 40 μm, differs conspicuously from *C. lamprauges* in size and by the formation of numerous small globules inside ripe zygospores 	Present in PDA medium. 12 to 18 μm, smaller than *C. incongruus* in size and has only a single large homogenous globule inside ripe zygospores 
Asexual conidia	Present. Globose 25 to 45 μm in diameter with roughly rounded apiculate basal papillae in mature conidia.	Present. Globose 16 to 34 μm in diameter with sharply pointed apiculate basal papillae in mature conidia.	Present. Globose to obovoid 15 to 22 μm in diameter with rounded apiculate basal papillae in mature conidia.
Replicative conidia similar to that in Fig. 2D.	Present. Similar to those reported in *C. incongruus* and *C. lamprauges*.	Present. Similar to those reported in *C. coronatus* and *C. lamprauges*.	Present. Similar to those reported in *C. coronatus* and *C. inconguus*.
Villose conidia 	Present. Few isolates recovered from mammals do not develop these conidia. The absence of zygospores on PDA could be of help in such cases	Absent	Absent
Multireplicative conidia 	Present, especially in water agar cultures	Present in some strains. Very similar to those reported in *C. coronatus*	Absent (this study and previous reports 5, 10)

FIG. 4. Microscopic differences between the three known mammalian pathogenic *Conidiobolus* species recovered in culture, based on data from references 5, 6, 10, 13, and 33 and from this study. PDA, potato dextrose agar.

incongruus in a clinical specimen from an infected white-tailed deer. While the latter approach is important in determining the etiologic agent in the absence of culture, the results have to be interpreted with caution. BLAST analysis of entire or partial rDNA or DNA coding sequences, although informative, could produce misleading data (4). The use of phylogenetics is a better approach for the proper placement of DNA sequences obtained from culture or infected tissue sections in the tree of life. Several studies using phylogenetic analysis of rDNA and protein-coding sequences have validated the classical taxonomic placement of some zygomycetes and have challenged the positions of various members within the Mucorales and Entomophthorales (11, 34, 36). Our phylogenetic study showed two well-supported sister groups in the genus *Conidiobolus*, data in agreement with those published by others (34, 36). The three DNA sequences from sheep that were evaluated clustered with two *C. lamprauges* sequences from the database, and together with *C. osmodes*, *C. thromboides*, *C. pumilus*, and *C. rhysosporus*, they formed a sister taxon to *C. coronatus*, *C. incongruus*, and the other two *Conidiobolus* species used in this study. In addition, the phylogenetic data in our study are in agreement with the phenotypic findings reported for the isolates investigated. The nucleotide differences encountered in the DNA sequences in this study could be used to build specific primers for the future identification of pathogenic *Conidiobolus* species from clinical samples.

REFERENCES

1. **Boabaid, F. M., E. V. Ferreira, L. P. de Arruda, N. D. Gasparetto, R. L. de Souza, M. C. Silva, V. Dutra, L. Nakazato, and E. M. Colodel.** 2008. Conidiobolomicose em ovinos no Estado de Mato Grosso. Pesq. Vet. Bras. **28:**77–81.
2. **Busapakum, R., U. Youngchaiyud, G. Segretain, and H. Fromentin.** 1983. Disseminated infection with *Conidiobolus incongruus*. Sabouraudia **21:**323–330.
3. **Carrigan, M. J., A. C. Small, and G. H. Perry.** 1992. Ovine nasal zygomycosis caused by *Conidiobolus incongruus*. Aust. Vet. J. **69:**237–240.
4. **Castresana, J.** 2000. Selection of conserved blocks from multiple alignments for their use in phylogenetic analysis. Mol. Biol. Evol. **17:**540–552.
5. **Drechsler, C.** 1953. Three new species of *Conidiobolus* isolated from leaf mold. J. Wash. Acad. Sci. **43:**29–43.
6. **Drechsler, C.** 1960. Two new species of *Conidiobolus* found in plant detritus. Am. J. Bot. **47:**368–377.
7. **French, R. A., and C. D. Ashworth.** 1994. Zygomycosis caused by *Conidiobolus coronatus* in a llama (*Lama glama*). Vet. Pathol. **31:**120–122.
8. **Gargas, A., and P. T. DePriest.** 1996. A nomenclature for fungal PCR

primers with examples from intron-containing SSU rDNA. Mycologia **88:** 745–748.
9. **Hernandez, M. J., W. Landaeta, B. N. Salazar, J. Vargas, and A. J. Rodriguez-Morales.** 2007. Subcutaneous zygomycosis due to *Conidiobolus incongruus*. Int. J. Infect. Dis. **11:**468–470.
10. **Humber, R. A., C. C. Brown, and R. T. Kornegay.** 1989. Equine zygomycosis caused by *Conidiobolus lamprauges*. J. Clin. Microbiol. **27:**573–576.
11. **Keeling, P. J.** 2003. Congruent evidence from α-tubulin and β-tubulin gene phylogenies for a zygomycete origin of microsporidia. Fungal Genet. Biol. **38:**298–309.
12. **Ketterer, P. J., M. A. Kelly, M. D. Connole, and L. Ajello.** 1992. Rhinocerebral and nasal zygomycosis in sheep caused by *Conidiobolus incongruus*. Aust. Vet. J. **69:**85–87.
13. **Kwon-Chung, K. J., and J. E. Bennett.** 1992. Medical mycology, p.447–463. Lea & Febiger, Philadelphia, PA.
14. **Madson, D. M., A. T. Loynachan, S. Kariyawasam, and T. Opriessnig.** 2009. Systemic *Conidiobolus incongruus* infection and hypertrophic osteopathy in a white-tailed deer (*Odocoileus virginianus*). J. Vet. Diagn. Invest. **21:**167–170.
15. **Mendoza, L., and A. A. Alfaro.** 1985. Equine zygomycosis in Costa Rica. Mykosen **28:**545–549.
16. **Mendoza, L., L. Ajello, and M. R. McGinnis.** 1996. Infections caused by the oomycetous pathogen *Pythium insidiosum*. J. Mycol. Med. **6:**151–164.
17. **Migaki, G., and P. W. Blumer.** 1975. Case for diagnosis (on phycomycosis in a dolphin). Mil. Med. **140:**544.
18. **Moll, H. D., J. Schumacher, and T. R. Hoover.** 1992. Entomophthoramycosis conidiobolae in a llama. J. Am. Vet. Med. Assoc. **200:**969–970.
19. **Morris, M., M. Ngeleka, A. O. Adogwa, G. Lalla, G. St-Germain, and R. Higgins.** 2001. Rhinocerebral zygomycosis in a sheep. Can. Vet. J. **42:**227–228.
20. **Pritchard, R. C., D. B. Muir, K. H. Archer, and J. M. Beith.** 1986. Subcutaneous zygomycosis due to *Saksenaea vasiformis* in an infant. Med. J. Aust. **145:**630–631.
21. **Ribes, J. A., C. L. Vanover-Sams, and D. J. Baker.** 2000. Zygomycetes in human disease. Clin. Microbiol. Rev. **13:**236–301.
22. **Riet-Correa, F., A. F. M. Dantas, E. O. Azevedo, S. D. V. Simões, S. M. S. Silva, R. Vilela, and L. Mendoza.** 2008. Outbreaks of rhinofacial and rhinopharyngeal zygomycosis in sheep in Paraíba, northeastern Brazil. Pesq. Vet. Bras. **28:**29–35.
23. **Roy, A. D., and H. M. Cameron.** 1972. Rhinophycomycosis entomophthorae occurring in a chimpanzee in the wild in East Africa. Am. J. Trop. Med. Hyg. **21:**234–237.
24. **Santurio, J. M., J. S. Argenta, S. E. Schwendler, A. S. Cavalheiro, D. I. B. Pereira, R. A. Zanette, S. H. Alves, V. Dutra, M. C. Silva, L. P. Arruda, L. Nakazato, and E. M. Colodel.** 2008. Granulomatous rhinitis associated with *Pythium insidiosum* infection in sheep. Vet. Rec. **163:**276–277.
25. **Sharma, N. L., V. K. Mahajan, and P. Singh.** 2003. Orofacial conidiobolomycosis due to *Conidiobolus incongruus*. Mycoses **46:**137–140.
26. **Silva, S. M. M. S., R. S. Castro, F. A. L. Costa, A. C. Vasconcelos, M. C. S. Batista, F. Riet-Correa, and E. M. S. Carvalho.** 2007. Conidiobolomycosis in sheep in Brazil. Vet. Pathol. **44:**314–319.
27. **Steiger, R. R., and M. A. Williams.** 2000. Granulomatous tracheitis caused by *Conidiobolus coronatus* in a horse. J. Vet. Intern. Med. **14:**311–314.
28. **Stephens, C. P., and J. A. Gibson.** 1997. Disseminated zygomycosis caused by *Conidiobolus incongruus* in a deer. Aust. Vet. J. **75:**358–359.
29. **Tabosa, I. M., F. Riet-Correa, V. M. Nobre, E. O. Azevedo, J. L. Reis-Júnior, and R. M. Medeiros.** 2004. Outbreaks of pythiosis in two flocks in northeastern Brazil. Vet. Pathol. **41:**412–415.
30. **Tamura, K., J. Dudley, M. Nei, and S. Kumar.** 2007. MEGA4: Molecular Evolutionary Genetics Analysis (MEGA) software version 4.0. Mol. Biol. Evol. **24:**1596–1599.
31. **Temple, M. E., M. T. Brandy, K. I. Koranyi, and M. C. Nahata.** 2001. Periorbital cellulitis secondary to *Conidiobolus incongruus*. Pharmacotherapy **21:**351–354.
32. **Thompson, J. D., D. G. Higgins, and T. J. Gibson.** 1994. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. Nucleic Acids Res. **22:**4673–4680.
33. **Tucker, B. E., and R. K. Benjamin.** 1978. *Conidiobolus coronatus*, p. 157–158. *In* M. S. Fuller (ed.), Lower fungi in the laboratory. Department of Botany, University of Georgia, Athens, GA.
34. **Voigt, K., E. Cigelnik, and K. O'Donnell.** 1999. Phylogeny and PCR identification of clinically important zygomycetes based on nuclear ribosomal-DNA sequence data. J. Clin. Microbiol. **37:**3957–3964.
35. **Walsh, T. J., G. Renshaw, J. Andrews, J. Kwon-Chung, R. C. Cunnion, H. I. Pass, J. Taubenberger, W. Wilson, and P. A. Pizzo.** 1994. Invasive zygomycosis due to *Conidiobolus incongruus*. Clin. Infect. Dis. **19:**423–430.
36. **White, M. M., T. Y. James, K. O'Donnell, M. J. Cafaro, Y. Tanabe, and J. Sugiyama.** 2006. Phylogeny of the Zygomycota based on nuclear ribosomal sequence data. Mycologia **98:**872–884.
37. **Zamos, D. T., J. Shumacher, and J. K. Loy.** 1996. Nasopharyngeal conidiobolomycosis in a horse. J. Am. Vet. Med. Assoc. **208:**100–101.